BioBrick cloning

Project: BioBricks
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THURSDAY, 9/15

In order to make our construct pAH12 (Mlra) and pAH05 (venus) biobrick compatitable PCR was conducted. Following PCR mixes were prepared

Table1

	A	B	C
1		pAH12	pAH05
2	Phusion hot start 2 Hifi mastermix	50	50
3	Forward primer	1 (ps13)	1 (ps15)
4	Reverse primer	1 (ps14)	1 (ps16)
5	Template DNA (50 ng)	0.5 ul	0.2 ul
6	H2O	47.5	47.8
7	Total volume	100 ul	100 ul

PCR was run with followind conditions

Table2

	A	B	C	D
1		time (s)	temperature (C)	Cycles
2	Initial denaturation	120	98	1
3	Denaturation	10	98	25
4	Annealing	30	60	25
5	Extension	20	72	25
6	Final extension	600	72	1

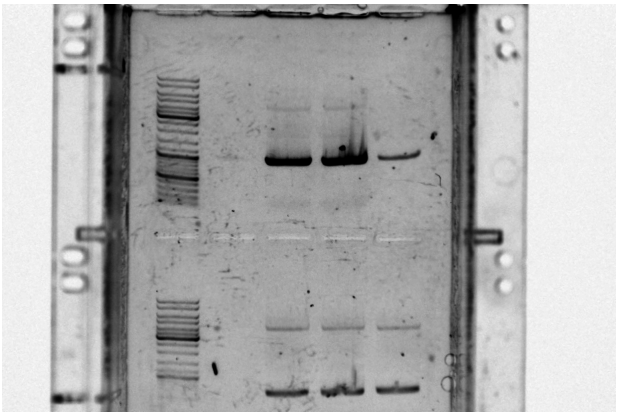
PCR products were run on agarose 0.7 % agarose gel. 20 ul of purple loading dye was pipeted into each product. Gel loading order: 1. row: ruler, empty, 3XpAH05. row 2: ruler, empty, 3XpAH12. Running was conducted with 100v. Gel was imaged but right sized band could not be observed.

09-15-2016 biobrick pcr.tif

FRIDAY, 9/16

It was noticed that primers were mixed in yesterdays PCR mixes and thus yesterdays PCR was conducted with similar mixes except in pAH05 forward primer was changed to ps13 and reverse primer to ps14. With pAH12 forward primer was changed to ps15 and reverse primer to ps16. PCR was run with similar condition as yesterday. PCR prducts were run on 0.7 % agarose gel with same loading order and running conditions. Gel was imaged and right sized bands could be observed. Loading order on the gel: 1. row: ruler, empty, 3X MlrA. 2. row: ruler, empty, 3X Venus

09-16-2016 biobrick pcr.tif



PCR products were cut off from the gel and products were purified from the gel with thermo fishers geneJET gel extraction kit and eluted for 30ul of elution buffer. DNA concentrations were measured from purified products with nanodrop and they were with Mlra 45.7 ng/ul and with venus 60.9 ng/ul.

THURSDAY, 9/22

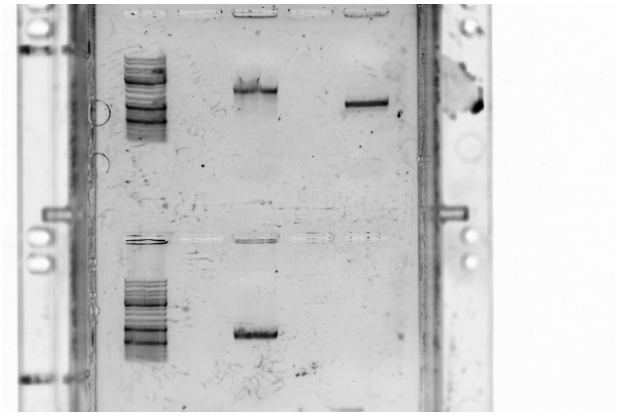
Restriction digest of plasmid backbone psb1c3 and venus and Mlra biobricks was conducted with following mastermixes (all volumes in ul)

Table3

	A	B	C	D
1		psb1c3	Mlra	Venus
2	FD EcoRI	1	1	1
3	FD Pst1	1	1	1
4	FD green buffer (10x)	3	3	3
5	Template DNA (200 ng)	8	4.4	3.3
6	H2O	17	20.6	21.7
7	Total volume	30	30	30

Digestion mixes were incubated 25min at 37 C and heat inactivated 5 min at 80 C. Digestion products were run on 0.7% agarose gel (loading order: 1. row: ruler, empty, psb1c3, empty, Mlra. 2. row: ruler, empty, Venus) with 100V. Gel was imaged and band were right sized.

Biobrick Restriction digest 22.9.2016.tif



Bands were cut off from the gel and purifed with Thermofisher geneJET gel extraction kit and eluted into 30 ul of elution buffer. Concentration of digestion products were measured with nanodrop and they were psb1c3: 5.6 ng/ul, Mlra: 6.6 ng/ul, Venus 10.8 ng/ul.

FRIDAY, 9/23

Ligation of digested plasmid backbone psb1c3 and inserts Venus and Mlra was conducted with following mixes (all units are in ul).

Table4

	A	B	C
1		Venus	Mlra
2	Vector DNA (20ng)	3.57	3.57
3	Insert DNA	2.06 (22.3 ng)	4.8 (31.7 ng)
4	10X T4 Ligase buffer	2	2
5	T4 DNA ligase	1	1
6	H20	11.37	8.63
7	Total volume	20	20

Ligation mixes wer incubated at room temperature for 10min and then heat inactivated in heat block at 65 C for 10min and then put on ice for transformation. Transformation was done with E.coli TOP10 competent cells with attached protocl (BACTERIAL TRANSFORMATION). Transformations were pipeted into 4 plates (total 8) containing chlormaphenicol.

SATURDAY, 9/24

Transformation plates of biobrick Mlra and venus were checked. All Venus plates contained many clonies but only 2 of the Mlra transformations only contained couple colonies. Plates were incubated at room temperature O/N.

SUNDAY, 9/25

Colony PCR of biobrick transformations was conducted with following PCR mastermix (all volumes in ul).

Table5

	A	B
1	Dreamtag green PCR mastermix (2x)	70
2	FW primer (VF2)	2.8
3	RW primer (VR)	2.8
4	H20	64.4
5	Total volume	140
6		

13 10ul PCR reactions were prepared from the mastermix (6 for both construct and 1 negative control). Small amount of bacteria colony was pipeted into each mastermix. PCR reactions were marked as colony 1-6. PCR was run with following conditions

Table6

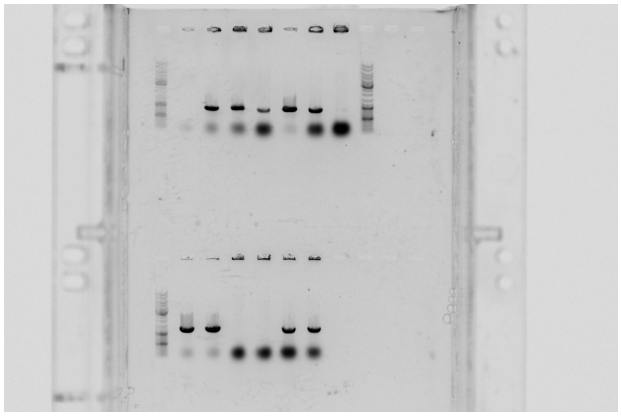
	A	B	C	D
1		time (s)	temperature (C)	Cycles
2	Initial denaturation	180	95	1
3	Denaturation	30	95	30
4	Annealing	30	50	30
5	Extension	90	72	30
6	Final extension	900	72	1
7	Hold	∞	4	1

PCR products were kept on PCR machine O/N and stored in fridge at the next morning

MONDAY, 9/26

Yesterdays PCR products were run on 0.7% agarose gel (loading order 1.row: ruler, NC, VenusX6 (1-6). 2.row: ruler, 6Xmlra(1-6)).

Colony pcr of biobricks 26.9.16.tif



Right size band was obtain from Venus colonies 1,2,4,5 and from Mlra colonies 1,2,5,6. 5 ml liquid cultures were prepared from these colonies into LB+chloramphenicol plates (50ug/ml). Liquid cultures were incubated at 37 C O/N.

TUESDAY, 9/27

Plasmid DNA was purified from yesterdays liquid culture colonies with Machery-nagel DNA, RNA and protein purification kit. The concentraions of purified plasmid was measured with nanodrop and they were Venus: C1: 669 ng/ul, C2: 98.7 ng/ul, C4: 86.8 ng/ul, C5: %9.5 ng/ul. Mlra: C1: 70 ng/ul, C2:68.0 ng/ul, C5: 58.6 ng/ul, C6: 84.5 ng/ul. DNA from colonies Venus 1,2,4 and from Mlra C1,C2,C6 were sent to sequencing.

SUNDAY, 10/2

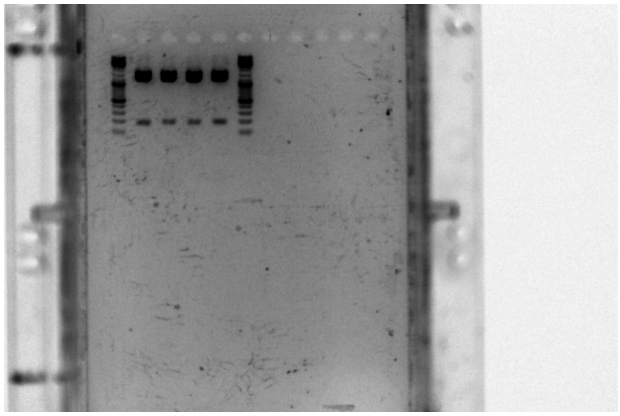
Sequencing results of biobricks arrived and Venus was fine but Mlra was not. Therefore, diagnostic restriction digest was conducted from Mlra biobrick plasmids (from clonies 1,2,5,6) with following mixes (NOTICE different enzyme used Spe1 run out so Pst1 was used instead which should results same sized bands, all units in ul).

Table7

	A	B	C	D	E
1		C1	C2	C5	C6
2	FD Nco1	0.5	0.5	0.5	0.5
3	FD Spel	0.5	-	-	-
4	FD Pst1	-	0.5	0.5	0.5
5	FD green buffer	2	2	2	2
6	Template DNA (500ng)	7.2	7.4	8.5	5.9
7	H20	9.8	9.6	8.5	11.1
8	Total volume	20	20	20	20

Mixes were incubated at 37 C for 10min and 10 ul of each mix was run on 2 % agarose gel with 100V (loading order: ruler, C1, C2, C5,C6).

2.10.16 Diagnostic digest of mlra biobrick.tif



Gel was imaged and contained right size plasmids. This result is in contradictory to the sequencing results of MlrA biobrick.

WEDNESDAY, 10/5

It was noticed that last time some of the plasmid purifications were done with wrong columns which would explaine why the Mlra sequencing gave poor result but diagnostic restriction digest gives correct result. Therefore, new plasmid purifcation will be conducted with right columns.
5ml liquid cultures of Mlra biobricks from colonies 1,2,5,6 were prepared into LB+chloramphenicol. Cultures were incubated at 37 C O/N.

THURSDAY, 10/6

From the Mlra liquid cultures the plasmid was purfied with Machery-nagel DNA, RNA and protein purification kit. Purified plasmid was sent to sequencing.

Bacterial Transformation

Introduction

Basic protocol for bacterial transformation from addgene (https://www.addgene.org/plasmid-protocols/bacterial-transformation/).

Materials

- Competent cells
- DNA to transform
- LB medium
- Appropriate antibiotics
- Agar plates containing appropriate antibiotics

Procedure

Transformation

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30min).
2. Take agar plates (containing the appropriate antibiotic) out of 4°C to warm up to room temperature or place in 37°C incubator.
3. Mix 1 to 5µl of DNA (usually 10pg to 100ng) into 20-50µL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.
4. Place the competent cell/DNA mixture on ice for 20-30min.
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 30-60 seconds (45sec is usually ideal, but this varies depending on the competent cells you are using).
6. Put the tubes back on ice for 2 min.
7. Add 250-500µl LB or SOC media (without antibiotic) and grow in 37°C shaking incubator for 45min.
8. Plate some or all of the transformation onto a 10cm LB agar plate containing the appropriate antibiotic. We recommend that you plate 50µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants. You can spread the transformation on even more plates, and plate the rest of the transformation the next day.
9. Incubate plates at 37°C overnight.